

Applicant : Cheng et al  
Serial No. :  
Filed : January 2, 2002  
Page : 6

Attorney's Docket No.: 08919-018002 / 03A-871028  
(DIV)

REMARKS

Applicants have amended the Specification to incorporate amendments that have been made to the parent application. Prompt examination of the present application, as amended, is respectfully requested.

Attached is a marked-up version of the changes being made by the current amendment.

Please apply any other charges to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 1-2-02

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**Version with markings to show changes made**

In the title:

Replace the title of the application with the following:

[METHOD OF CLEAVING A NUCLEIC ACID BULGE] NUCLEIC ACID BULGE-  
DETECTING AGENT

In the specification:

Insert the following paragraph beginning at page 1, line 1:

This application is a divisional application of U.S. Serial Number 09/302,334 filed April 30, 1999, pending issue.

Replace the paragraph beginning at page 7, line 18 with the following rewritten paragraph:

Note that the metal ion of each of the complexes of formula (I) adopts an [octehedral] octahedral coordination. For example, the X-ray crystal structure of cobalt(II)(hexaazacyclophane)(trifluoroacetate)<sub>2</sub>, i.e., Co<sup>II</sup>(HAPP)(TFA)<sub>2</sub>, reveals that the complex contains two labile axial TFA ligands, and two linked 1,10-phenanthroline moieties where all four pyridyl nitrogen atoms are locating on the same coordination plane. The average Co-N distance is approximately 1.86 Å. EPR spectrum of the Co<sup>II</sup> complex gave a g<sub>av</sub> value at 2.005-2.331 in methanol, indicating the presence of an octahedral Co<sup>II</sup> complex. When one equivalent of pyridine was added, it rapidly displaced one of the axial TFA ligands under ambient conditions, as monitored by EPR spectroscopy, suggesting that the TFA ligands are labile. The TFA ligands can also be readily substituted by water upon dissolution of the complex in aqueous buffer.

Also replace the paragraph beginning at page 13, line 2 with the following rewritten paragraph:

A 26-mer 5'-GCAGACTGAGCCTGGGAGCTCTCTGC-3' (SEQ ID No. 4) (D, Figure 1) was used as the DNA substrate. It was prepared according to the same procedures as described

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in Example 1. Note that substrate D only differs from substrate A in that its bulge contains one less base.

Further replace the paragraph beginning at page 13, line 13 with the following rewritten paragraph:

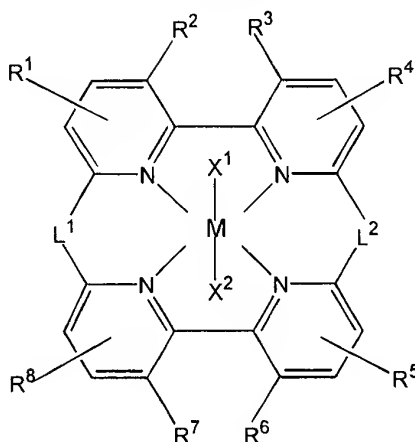
$\text{Co}^{\text{II}}(\text{HAPP})(\text{TFA})_2$  ( $0.6 \mu\text{M}$ ) was allowed to react under identical conditions as described above with a single-stranded 16-mer of the sequence 5'-GCCAGATCTGAGCCTG-3' (SEQ ID No. 2) (B, Figure 1) in the presence of  $\text{H}_2\text{O}_2$ . No specific cleavage was observed at the 5'-TCT-3' site, even when the concentration of the cobalt complex was increased by 20-fold. The single-stranded substrate was then allowed to anneal with a complementary DNA strand 5'-CAGGGCTCTCTGCC-3' (SEQ ID No. 3) to form a double-stranded DNA with a three-base bulge (C, Figure 1). When the  $\text{Co}^{\text{II}}$  complex was added to the double-stranded substrate, enhanced DNA cleavage was observed at the 5'-TCT-3' bulge. These results indicate that the  $\text{Co}^{\text{II}}$  complex serves as a DNA bulge-specific cleavage reagent without significant specificity towards the corresponding sequence in the single-stranded DNA.

In the claims:

Claims 17-27 have been cancelled.

Claims 1-16 have been reiterated as follows:

1. (Reiterated) A metal complex of the following formula:



wherein

each of  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ , and  $R^8$ , independently, is hydrogen, alkyl, alkoxy, hydroxyl, hydroxylalkyl, halo, haloalkyl, amino, aminoalkyl, alkylcarbonylamino, alkylaminocarbonyl, alkylcarbonyl, alkylcarbonylalkyl, alkoxycarbonyl, alkylcarbonyloxy, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; each of  $R^2$  and  $R^3$ , and  $R^6$  and  $R^7$ , independently, optionally joining together to form a cyclic moiety fused with the two pyridyl rings to which  $R^2$  and  $R^3$ , or  $R^6$  and  $R^7$  are bonded; the cyclic moiety, if present, optionally being substituted with alkyl, alkoxy, hydroxyl, hydroxylalkyl, halo, haloalkyl, amino, aminoalkyl, alkylcarbonylamino, alkylaminocarbonyl, alkylcarbonyl, alkylcarbonylalkyl, alkoxycarbonyl, alkylcarbonyloxy, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

each of  $L^1$  and  $L^2$ , independently, is  $-C(R^a)(R^b)-$ ,  $-O-$ ,  $-S-$ , or  $-N(R^c)-$ ; each of  $R^a$ ,  $R^b$ , and  $R^c$ , independently, is hydrogen, alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

M is a Co, Ni, Ru, Rh, Mn, Os, Ag, Cr, Zn, Cd, Hg, Re, Ir, Pt, or Pd ion; and

each of  $X^1$  and  $X^2$ , independently, is a labile ligand;

or a salt thereof.

2. (Reiterated) The metal complex of claim 1, wherein each of  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ , and  $R^8$ , independently, is hydrogen, alkyl, or alkoxy.

3. (Reiterated) The metal complex of claim 1, wherein each of  $R^2$  and  $R^3$ , and  $R^6$  and  $R^7$ , independently, join together to form a cyclic moiety; the cyclic moiety being benzene.

4. (Reiterated) The metal complex of claim 3, wherein the cyclic moiety is unsubstituted.

5. (Reiterated) The metal complex of claim 4, wherein each of  $R^1$ ,  $R^4$ ,  $R^5$ , and  $R^8$ , independently, is hydrogen, alkyl, or alkoxy.

6. (Reiterated) The metal complex of claim 5, wherein each of  $R^1$ ,  $R^4$ ,  $R^5$ , and  $R^8$ , independently, is hydrogen.
7. (Reiterated) The metal complex of claim 6, wherein each of  $L^1$  and  $L^2$ , independently, is  $-N(R^c)-$  where  $R^c$  is hydrogen.
8. (Reiterated) The metal complex of claim 7, wherein M is Co.
9. (Reiterated) The metal complex of claim 8, wherein  $X^1$  and  $X^2$ , independently, is trifluoroacetate.
10. (Reiterated) The metal complex of claim 9, wherein said complex is cobalt(II) (hexaazacyclophane) (trifluoroacetate).
11. (Reiterated) The metal complex of claim 1, wherein each of  $L^1$  and  $L^2$ , independently, is  $-S-$  or  $-N(R^c)-$ .
12. (Reiterated) The metal complex of claim 11, wherein each of  $L^1$  and  $L^2$ , independently, is  $-N(R^c)-$  where  $R^c$  is hydrogen.
13. (Reiterated) The metal complex of claim 1, wherein M is Co, Ru, or Mn.
14. (Reiterated) The metal complex of claim 13, wherein M is Co.
15. (Reiterated) The metal complex of claim 1, wherein  $X^1$  and  $X^2$ , independently, is  $H_2O$ , Cl, trifluoroacetate, or pyridine.
16. (Reiterated) The metal complex of claim 15, wherein  $X^1$  and  $X^2$ , independently, is trifluoroacetate.

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